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(54) Title: MEMBRANE FORMED BY AN ACRYLONITRILE-BASED POLYMER

$$-CH_2CH- \atop {\displaystyle | \atop \displaystyle C\equiv N}$$ (a)

(57) Abstract

A permselective graft polymer is disclosed that is formed by converting into intermediate reactive sites a portion of the cyano groups of a backbone polymer containing (a) units, and grafting polyalkylene oxide polymer chains to the backbone polymer through the reactive sites. Either the backbone polymer of a polymer resin or a permselective polymer membrane can be grafted. When a resin is used, it is formed into a permselective polymer membrane using known methods. The resulting permselective membrane can be formed into hollow fibers or flat sheets for the encapsulation of living cells. The encapsulated cells are then implanted into a patient in need of the biologically-active factors produced by the cells. The permselective graft polymer membrane exhibits good molecular diffusion with minimal protein adsorption.

MEMBRANE FORMED BY AN ACRYLONITRILE-BASED POLYMER

REFERENCE TO RELATED APPLICATION
This is a continuation-in-part application of patent
application U.S.S.N. 08/053,899, filed 27 April 1993.

5   Field of the Invention
The technical field of this concerns graft polymers
and graft polymer membranes useful for the
encapsulation of living cells which produce
biologically active factors. More particularly, the
10   present invention relates to graft homopolymers and
copolymers of poly(acrylonitrile) and polyalkylene
oxide. The graft polymer membranes are permselective
and allow proteins produced by the encapsulated cells
to be readily diffused through the membrane.

15   Background of the Invention
Selectively permeable membranes have been used to
encapsulate cells which secrete biologically-active
factors useful for the treatment of various diseases
and disorders. Typically, the cells are loaded into
20   the membranes which are in the form of hollow fibers
or between two flatsheets in the form of a sandwich.
The fibers are then sealed at the ends to form
"macrocapsules". The encapsulated cells are
implanted into a patient in need of the biologically-
25   active factors produced by the cells. Macrocapsules

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offer the advantage of easy retrievability, an
important feature in therapeutic implants.

An example of macrocapsules can be found in U.S.
Patent No. 4,892,538, which describes the
5   encapsulation of neurotransmitter-secreting cells
which are implanted into a patient having a
neurotransmitter-deficiency disease.

U.S. Patent No. 5,158,881 also discloses methods and
systems for encapsulating cells which produce
10 biologically-active factors. The cells are
encapsulated with a semipermeable polymeric membrane
by co-extruding an aqueous cell suspension and a
polymeric solution through a common port to form a
tubular extrudate having a polymeric outer coating
15 which encapsulates the cell suspension. Cells can
also be loaded into pre-formed hollow fiber
membranes.

Typically, the semipermeable membranes used to
encapsulate cells are formed from polymeric materials
20 such as acrylic copolymers, polyvinylidene fluoride,
polyurethane isocyanates, polyalginate, cellulose
acetate, polysulfone, polyvinyl alcohols,
polyacrylonitrile and mixtures or derivatives
thereof. Poly(acrylonitrile-co-vinyl chloride)
25 (PAN/PVC) is one of the polymers used to make
implantable membranes because it can easily be made
into permselective membranes that allow easy
transport of nutrients and greatly reduce transport
of immuno-molecules. These membranes can be made
30 with a wide variety of wall thicknesses and
morphologies. PAN/PVC is moderately hydrophilic and
is non-toxic to cells.

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While these materials have the capability of being
formed into permeable-selective, biocompatible
membranes, there exists the need to further improve
the characteristics of the membranes to increase
5   their utility for macroencapsulation purposes. One
shortcoming of some polymeric membranes is that
proteins secreted from the encapsulated cells and
proteins from the patient, tend to adsorb to them,
thus decreasing the diffusion rate of the protein to
10   the patient and hence the efficiency of the implant.

Various modifications have been made to polymeric
materials to change their characteristics and to
improve their usage for particular therapeutic
applications. For example, U.S. Patent No. 4,871,785
15   to Froix et al. describes hydrogel contact lens
compositions which are modified to contain
significant amounts of a cross-linking material such
as polyethylene oxide. The modification results in a
lens having decreased protein adsorption.
20   Allmer et al. have grafted polyethylene glycol (PEG)
and heparin onto polymer surfaces to inhibit protein
adsorption and to prevent surface activated blood
clotting [J. of Polymer Sci. Vol 28:176-183 (1990)].
Miyama et al. describe graft copolymers having
25   improved antithrombogenicity after being heparinized
[J.Biomed Mater. Res., Vol. 11:251-265 (1977)]. U.S.
Patent No. 4,424,311 to Nagaoka et al. describes an
antithrombogenic biomedical material comprising a
polymer having a polyethylene oxide unit. U.S.
30   Patent No. 4,965,112 to Brinkman et al. describes a
method for applying polyethylene oxide coating to
polyether-urethane molded articles such as catheters
in order to improve blood-compatibility. Published
PCT application PCT/US91/07051 describes grafting
35   poly(ethylene oxide) onto microcapsules made of
polycationic polymers such as poly(l-lysine). Fane

et al. disclose that treating various ultrafiltration
membranes with nonionic surfactants can enhance flux
of protein solutions [Desalination, Vol. 53:37-55
(1985)].

5   Summary of the Invention
It is an object of the present invention to provide a
biocompatible, permselective hollow fiber membrane
which exhibits good molecular diffusion with minimal
protein adsorption.

10   It is a further object of the invention to provide
graft polymer and copolymer membranes having
functional surface groups by which additional
modifications can be performed to further increase
the utility and biocompatibility of the membranes.

15   These objects are accomplished by the graft polymer
and permselective graft polymer membranes of the
present invention. The graft polymer is formed by
converting into intermediate reactive sites a portion
of the C≡N (cyano) groups of a backbone polymer
20   containing —CH₂CH— units.
                      |
                     C≡N

Polyalkylene oxide polymer chains are then grafted to
the backbone polymer through the reactive sites.

25   In one embodiment, the graft polymer is formed by
copolymerizing acrylonitrile monomer and vinyl
chloride monomer to form a backbone copolymer. The
backbone copolymer includes the following groups
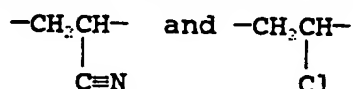along the copolymer chain:
30   —CH₂CH—   and —CH₂CH—
             | |
            C≡N   Cl
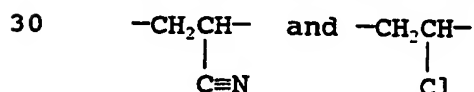
where a portion of the —C≡N (cyano) groups have been
converted into intermediate reactive sites.

Polyalkylene oxide polymer chains are then grafted to
the backbone copolymer through the reactive sites.

In one embodiment, the polyalkylene oxide polymer
chains are used to modify the backbone polymer of a
5   permselective polymer membrane to form a
permselective graft polymer membrane. In another
embodiment, the polyalkylene oxide polymer chains are
used to modify the backbone polymer of a polymer
resin to form a graft polymer resin. The resin is
10   then formed into a permselective graft polymer
membrane using methods known in the art.

The invention also comprises a method for forming a
graft polymer where acrylonitrile monomer and vinyl
chloride monomer are copolymerized to form a backbone
15   copolymer including the following groups:

$$-CH_2CH- \quad \text{and} \quad -CH_2CH-$$
$$|\phantom{CH_2CH- \quad \text{and} \quad -CH_2CH} |$$
$$C{\equiv}N \phantom{CH- \quad \text{and} \quad -CH_2} Cl$$

A fraction of the cyano groups are then converted
20   into intermediate reactive sites and polyalkylene
oxide polymer chains are grafted to the backbone
copolymer through the reactive sites.

Brief Description of the Drawings
The invention can be more fully understood from the
25   following description when read together with the
accompanying drawings in which:
FIG. 1 shows the acid hydrolysis of a
poly(acrylonitrile-co-vinyl chloride) (PAN/PVC)
backbone, wherein x and y represent the ratio of
30   acrylonitrile to vinyl chloride, respectively. The
hydrolyzed PAN/PVC copolymer is then derivatized with
polyethylene glycol amine.
FIG. 2 shows the sodium borohydride reduction of a
PAN/PVC backbone, wherein x and y represent the ratio

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of acrylonitrile to vinyl chloride, respectively.
The reduced PAN/PVC copolymer is then derivatized
with polyethylene glycol succinimide.

FIG. 3 shows the ^{1}H NMR spectrum for PAN/PVC

5   copolymer.

FIG. 4 shows the ^{1}H NMR spectrum for reduced PAN/PVC
copolymer.

FIG. 5 shows the ^{1}H NMR spectrum for SC—PEG—8000
coupled to reduced polymer.

10 **FIG. 6** shows the ^{1}H NMR spectrum for hydrolyzed
PAN/PVC copolymer.

FIG. 7 shows the ^{1}H NMR spectrum for the hydrolyzed
polymer coupled to M—PEG—NH$_2$

Detailed Description of the Invention

15 The inventors have found that a modification of
poly(acrylonitrile) (PAN)-based hollow fibers with an
activated poly(alkylene) oxide (PAO) results in a
permselective biocompatible membrane having decreased
protein adsorption. The membrane of the present

20 invention is a graft polymer that exhibits
considerably less protein adsorption than the
copolymer of polyvinyl chloride and polyacrylonitrile
(PAN/PVC). Without being limited to a particular
theory, PAO is thought to exclude a volume about the

25 surface, thereby inhibiting proteins from "arriving"
at or adsorbing to the membrane. The un-modified
membrane is insoluble in aqueous solution whereas
PAO, as are proteins, is soluble in aqueous solution.
The PAO chain is thought to extend from the insoluble

30 surface into the aqueous solution, thereby inhibiting
protein adsorption.

In addition to decreasing protein adsorption of the
membrane, the grafted PAO provides hydroxy groups
onto which additional molecules can be attached. For

35 example, it may be desirable to attach various

proteins, cellular adhesion molecules, anti-cellular
adhesion molecules, etc. Also, enzymes could be
attached that bind or inactivate viruses or IgG, thus
improving the biocompatibility of the permselective
5 membrane.

As used herein, the term "permselective" is used to
describe a biocompatible membrane that allows the
passage of substances up to a predetermined size, but
prevents the passage of larger substances. More
10 specifically, the membrane is produced in such a
manner that it has pores or voids of a predetermined
range of sizes; as a result, the vehicle is
permselective. The molecular weight cutoff (MWCO)
selected for a particular membrane will be determined
15 in part by the type and extent of immunological
rejection it is anticipated will be encountered after
the membrane is implanted and in part by the
molecular size of the largest substance to be allowed
to pass into and/or out of the vehicle. For example,
20 membranes can be formed which allow passage of
molecules up to about the size of Clq, a component of
complement (about 400 kD), a protein required for the
assembly of the cytolytic complement attack complex.
In this instance, substances smaller than Clq can
25 pass freely. It is also possible to form
permselective membranes which allow passage of
molecules up to about the size of immunoglobulin G
(about 150 kD) and exclude larger molecules.
Further, permselective membranes or hydrogels which
30 allow passage of molecules up to about the size of
immunoglobulin M (about 1,000 kD) can be used; only
very large substances, such as cells, will be
excluded in this embodiment.

The permselective membranes can be formed into hollow
35 fibers or flat sheets. A hollow fiber membrane is an

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annulus consisting of an inner cylindrical surface, a
wall structure for support, and an outer cylinder
surface. One or both of the surfaces can be
selective for molecules of varied molecular weight.

5   A flatsheet is a planar composition of a hollow
fiber.

In one embodiment, a membrane formed by
copolymerizing acrylonitrile monomer and vinyl
chloride monomer to form a backbone copolymer of

10 PAN/PVC is obtained. However other PAN-based
copolymer membranes can also be used including but
not limited to PAN-co-vinylidene chloride, PAN-co-
acrylic acid, PAN-co-butadiene-styrene, PAN-co-
butadiene, PAN-co-vinyl acetate, PAN-co-4-vinyl

15 pyridine, PAN-co-butadiene-co-acrylic acid, and PAN-
co-maleic anhydride. Also, PAN homopolymer, and PAN-
based block copolymer membranes can be used as well.
As used herein, the term "copolymer" includes
terpolymers. It also includes random copolymers and

20 block copolymers. Methods known in the art can be
used to form PAN copolymer membranes, including U.S.
Patent No. 2,763,631 to Coover et al., U.S. Patent
No. 2,420,330 to Shriver et al., and U.S. Patent No.
4,334,046 to König et al., which describe PAN/PVC and

25 are incorporated herein by reference.

Once the PAN-based polymer membrane is obtained, a
portion of the —C≡N (cyano) groups of the backbone
are converted into intermediate reactive sites.
Polyalkylene oxide polymer chains (e.g. polyethylene

30 glycol or polypropylene glycol, preferably the
former) are then grafted to the backbone copolymer
through the reactive sites. The polyalkylene oxide
polymer chains used in the present invention are
substantially water soluble. The reactive sites are

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groups to which the PAO terminal groups may react
within a single reaction step.

In one method, the reactive sites of the PAN/PVC
backbone membrane are formed by hydrolysis of the
5   cyano groups into a chemically available carboxyl
group. The carboxyl groups are reacted with an amine
terminal group on a precursor polyalkylene oxide
(PAO) polymer chain to form the graft copolymer. For
example, in FIG. 1, the carboxyl groups of the
10   PAN/PVC backbone are formed by acid hydrolysis. They
are then reacted with polyethylene glycol-amine to
produce a graft copolymer. FIG. 1 depicts the acid
hydrolysis of a PAN/PVC copolymer wherein the ratio
of acrylonitrile to vinyl chloride is 45:55.
15   However, other ratios of acrylonitrile to vinyl
chloride may also be used.

The graft copolymers of the present invention can
also be formed by reduction of PAN/PVC. The reactive
sites are formed by reduction of the cyano groups
20   into amine groups. The applicants have found that
sodium borohydride reduction of the copolymer
produces amino groups that can be used to bind an
activated PAO such as a succinimidyl carbonate-
derivatized PAO. This is shown in FIG. 2, where the
25   amine groups are then reacted with polyethylene
glycol succinimide. Activated PAOs can be
commercially obtained, for example, from
Polysciences, Inc., Warrington, PA. and Shearwater
Polymers, Huntsville, AL.

30   Another method for producing the graft copolymers of
the present invention, involves reacting the chloride
groups of PVC with thiol urea to produce thiol groups
on PVC (PVC-SH) and then reacting epoxide terminated

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PAO with PVC—SH to produce grafted PAO units on the
PVC portion of PAN/PVC fibers.

PAN/PVC—PEG can also be produced by reacting amine-
terminated polyethylene oxide (PEO—NH$_2$) with 4-
5   fluoroaniline amino azide to produce PEO
functionalized with a nitrene group (i.e. PEO-phenyl
azide).   The nitrene reacts photochemically and
randomly by insertion into C—H bonds, thereby
functionalizing the PAN/PVC backbone randomly.

10   Proton NMR is a suitable analytical procedure for
quantifying the amount of PAO attached to the
PAN/PVC.   The graft copolymer can also be
characterized by other spectroscopic methods such as
attenuated total reflectance fourier transform
15   infrared spectroscopy (ATR FTIR) and x-ray
photoelectron spectroscopy (XPS).   By ATR FTIR, it is
evident that the PAN nitrile group is hydrolyzed via
an amide group.   Although all nitrile groups are not
completely hydrolyzed, they react with PEG-amine.   As
20   shown by ATR FTIR, the amide peak area increases
whereas the carboxylic acid peak decreases.   In
addition, the presence of a (C—O) peak at 1100 cm^{-1}
is evident in the IR spectrum, indicative of the PEG.
Reduction of the nitrile group of PAN/PVC also gives
25   rise to an amide peak of unknown origins.   However,
after coupling with the PEG-succinimide, a (C—O) peak
at 1100 cm^{-1} is present and a decrease of the (N—H)
peak and an increase in the amide peak are evident.
By proton NMR, the reduction of PAN/PVC and the
30   grafting of PEG is evident from chemical shifts.

In another embodiment of the invention, a polymer
resin itself may first be modified, and then a
membrane may be formed from the modified resin using
methods known in the art such as the methods

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described by H. Strathmann in Material Science of
Synthetic Membranes, "Production of Microporous Media
by Phase Inversion Processes," pp. 165-195. American
Chemical Society (1985). Basically, the resin is
5   modified using the same chemistry as is described
above for the PAO modification of the surface of a
polymer membrane. The term "resin" as used herein
refers to the bulk form of a polymer and includes
resins that are in powder, liquid, or pellet forms.

10   Protein adsorption involves the interaction of
proteins in solution with a solid (insoluble)
surface. The interaction can be described as either
chemisorption (e.g. ionic) or physisorption (e.g.
hydrophobic). PAO modification of the surface
15   inhibits protein adsorption which is a surface
phenomenon. While the surface of a non-porous film
is well defined, that of a phase inversion membrane
is not. For the latter, the surface that is exposed
to protein includes the surface of the pores that run
20   through the membrane. Modification of a polymer
resin prior to membrane formation is a method that
may allow for more even modification of the
interstices of the pores than methods that involve
surface modification of the membrane.

25   **Examples 1-4: Formation of graft copolymer membranes**
In order to exemplify but not limit the scope of the
invention, the following examples are provided to
show various procedures useful for attaching PAO to a
PAN/PVC copolymer.
30   **Example 1**
Reduction of polymer with NaBH₄
Copolymer (sheets or tubes) was suspended in a 10%
aqueous solution of NaBH₄ (0.7g in 7ml water) for 48h
at room temperature, then washed with water and 95%
35   ethanol.

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Example 2
Coupling of SC-PEG with reduced polymer

Reduced copolymer was stirred with 7 ml of sodium
phosphate buffer pH 8.0. SC-PEG 20,000 (0.7g) was
5   added and stirring continued at 40°C for 2h. The
reacted polymer was then washed with water and 95%
ethanol and left in 95% ethanol at 40°C for 48h. The
sample was then washed with water. A similar
experiment was conducted with SC-PEG 8,000 except
10 that the reaction was conducted at room temperature
and all washes were done with water.

Example 3
Hydrolysis of polymer with HCl-NaOH

Copolymer was stirred for 48h in 10 ml of
15 concentrated HCl, then washed with water. The
polymer was then added to 10 ml of 10M NaOH solution
and stirred for 48h. The product was washed with
water until neutral then treated with 5% oxalic acid
for 10 minutes and again washed with water and 95%
20 ethanol.

Example 4
Coupling of MPEG-NH$_2$ (5000) to hydrolyzed polymer

M-PEG-NH$_2$ (0.7g), N-(3-dimethylaminopropyl)-ethyl
carbodiimide (0.28g) and hydrolyzed polymer were
25 stirred in water (7ml). The pH of the mixture was
adjusted to 4.5 with dilute HCl. The mixture was
heated to 40°C for 5h, then washed with water and 95%
ethanol and kept in 95% ethanol at 40°C for 48h. A
final wash with water was performed. A companion
30 experiment without ethanol washing was performed.

The products prepared from Examples 1-4 were examined
with ^{1}H NMR. The NMR spectra were obtained by
dissolving 4 to 5 mg of the polymer samples in 0.5 ml
of d$_6$-acetone. Spectra were obtained on a 200 MHz
35 Bruker machine. All peaks were relative to acetone.

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The NMR spectrum for the starting PAN/PVC copolymer
is shown in FIG. 3. Note the H—C—Cl peak at 4.43 ppm
(peak A), H—C—CN peak at 3.39 ppm (peak B), water at
2.87 ppm, —CH$_2$— at 2.44 ppm, and acetone at 2.06 ppm.

5   The ratio of peak A to peak B is 55/45, thus
indicating that the polymer contains 55% vinyl
chloride to 45% acrylonitrile.

The spectrum for reduced polymer as prepared in
Example 1 is shown in FIG. 4. The A/B ratio

10 increases to 62/38 (as expected for borohydride
reduction of the —CN group and consequent decrease in
size of the H—C—CN peak).

The spectrum for SC—PEG-8000 coupled to reduced
polymer, as prepared in Example 2, is shown in FIG.

15 5. This clearly shows the incorporation of PEG,
which has a peak at 3.60 ppm. Addition of all the
areas and division into the PEG area (120) shows 1.8%
PEG.

The spectrum for the hydrolyzed polymer, as prepared

20 in Example 3, is shown in FIG. 6. This shows an A/B
ratio very similar to the starting material. This
indicates that the new —CH—CO$_2$— and —CH—CONH$_2$ peaks
are close to the —CH—CN peak.

FIG. 7 shows the spectrum for the hydrolyzed polymer

25 coupled to M—PEG—NH$_2$, as prepared in Example 4.
There is a small PEG peak at 3.61 ppm. This spectrum
shows that the hydrolytic pathway is less effective
at incorporating PEG into PAN/PVC than the reduction
pathway; however, hydrolysis conditions could be

30 optimized to provide more incorporation of the PEG if
desired.

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Examples 5-9: Measurement of protein adsorption, diffusion, and nMWCO

Example 5
Flatsheet Adsorption Study

5 An FTA solution was prepared by dissolving 9.23 g of FTA and 0.5g of sodium azide into 1 l of water. A 5% BSA solution was prepared by dissolving 5 g BSA into 100 ml of the FTA solution with stirring. Three flatsheets were mounted in 3 chambers with BSA

10 solution on one side and FTA on the other at room temperature. The permselective layer was facing the FTA solution. The solution on either side was monitored by UV absorbance for BSA. When the BSA had diffused across the membrane to an equilibrium value,

15 the flatsheets were removed, washed 5 times with FTA, and assayed by the BCA test for protein adsorption. The flatsheets exposed to the BCA test were 1 cm in diameter. The amount of protein adsorbed was estimated from a calibration curve (μg/ml) which was

20 then converted to μg/cm^2 by dividing by the approximate surface area of the flatsheet.

Example 6
Fiber Adsorption Study

Three 1 cm long fibers of each type were immersed in

25 5% BSA in 6-well plates and incubated at 37°C for 3 days. The fibers were removed and washed 5 times with FTA over 1 h and then assayed by the BCA test for protein adsorption. The amount of adsorbed protein was calculated by comparison to a calibration

30 curve for BSA (μg/ml) which was then converted to μg/cm^2 by multiplying by 0.1 ml and then dividing by the approximate surface area of the fiber (πdl).

Example 7
BCA Test

35 A standard BCA test procedure was followed. One fiber or flatsheet not exposed to any protein

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solution was used as the control and the blank
solution consisted of FTA alone. Three fibers or
flatsheets were exposed to BCA reagents for 2 h at
room temperature and then the UV absorbance was read
5   on a microplate reader using Softmax hardware.

Example 8
Diffusion Test

Fibers 2.5 cm long were glycerinized and then fitted
with 0.5 cm tecoflex using polyurethane glue. The
10   fibers were then deglycerinized by immersion in FTA
for at least 2 days. The fibers were quickly dried
and then 40 μl of 3 mg/ml of α-chymotrypsinogen was
inserted via pipet into the lumen of a fiber. The
fiber was sealed by heating the tecoflex end and then
15   rinsed in FTA for approximately 2 minutes before
inserting the fiber (vertically) into 6 ml of FTA in
a 15 ml conical centrifuge tube. Diffusion was
monitored by UV absorption at 281 nm over 24 h.

Example 9
20   ### Determination of nominal molecular weight cutoff
(nMWCO)

The nMWCOs of fibers 2 cm long were characterized by
the MWCO test to determine what size molecules will
cross the membranes. The extent to which a given
25   molecular weight molecule will cross a membrane is
measured by the rejection coefficient, R, such that R
$= 1 - C_p/C_r$, where C_p is the concentration in a
solution of a molecular weight molecule that crosses
a membrane and C_r is the concentration of the
30   molecule that is excluded by the membrane. A
membrane's nMWCO is the molecular weight of a
molecule with R $\geq$ 90%. MWCO is measured under
convective (pressurized) conditions 5 $\pm$ 1 psig) and
ambient room temperature (18-28°C). Normally 10-20
35   fibers are measured simultaneously (cartridge test)

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but individual fibers can be measured by making a
single fiber coil (coil test).

The data obtained from Examples 5-9 is summarized in
Table I.

5   TABLE I

Sample HF = hollow fiber FS = flatsheet	Protein Adsorption (BSA) ($\mu g/cm^2$)	MWCO BSA Permeability
PAN/PVC (HF)	17.1 ± 1.5	95%
PAN/PVC-PEG-NH$_2$ (5k) (HF)	12.0 ± 1.0	95%
PAN/PVC-PEG-NH$_2$ (20k) (HF)	11.7 ± 0.8	
PAN/PVC (FS)	1.3 ± 0.6	n/a
PAN/PVC-PEG-NH$_2$ (5k) (FS)	0.6 ± 0.0	n/a
PAN/PVC-PEG-SC (8k) (FS)	0.6 ± 0.0	n/a
PAN/PVC-PEG-SC (20k) (FS)	0.5 ± 0.0	n/a

As can be seen from Table I, grafting PEG-NH$_2$ to
PAN/PVC decreased the amount of protein adsorbed
relative to non-grafted PAN/PVC. However, the
molecular weight cut-off did not changed appreciably
after the surface modification reaction with PEO. It
is likely that polyethylene oxide-functionalized
PAN/PVC enhances apparent diffusion by decreasing the
amount of protein that is adsorbed during diffusion.
The molecular weight cut-off data indicates that the
pore structure is not adversely affected by the
chemistry involved in grafting polyethylene oxide.

Example 10:   Insulin passivated fibers

Fibers were prepared as in Examples 1-4 above. Then
the procedure of Example 8 was followed, however
after degylcerinization the fibers were soaked in an
insulin containing buffer @ 37° C for 16 hours. The

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results of the diffusion test are shown in Table II
below.

TABLE II

	Sample	Dmc Insulin-Treated (cm²/s x 10⁸) (n = 3)	Dmc Not Treated (cm²/s x 10⁸ (n = 3)
5	PAN/PVC	3.0	0.3
	PAN/PVC-PEO-NH₂ (5k)	1.0	0.9
	PAN/PVC-PEO-NH₂ (20k)	2.2	1.6
	PAN/PVC-PEO-SC (8k)	0.7	0.8
10	PAN/PVC-PEO-SC (20k)	0.7	0.6

Typically, insulin passivation affects an increase in
the diffusion coefficient (Dmc) of PAN/PVC. However,
insulin passivation has little affect on the
diffusion coefficient of PEO-grafted PAN/PVC. Given
15   the presumption that insulin passivates the PAN/PVC
membrane by adsorption, it is likely that only an
insignificant amount of insulin adsorbs to PEO-
PAN/PVC. Thus, PEO effectively inhibits protein
adsorption and thus insulin passivation does not
20   affect the diffusion coefficient of PEO-PAN/PVC.

Example 11: Biocompatibility of PEO-PAN/PVC
In vivo biocompatibility of PEG-grafted PAN/PVC was
assessed by implanting fibers into the brain and sub-
cutaneous sites of Lewis rats (about 250g). Fibers
25   with and without PEG were implanted. The fibers were
sterilized by immersion in 70% ethanol overnight and
then in sterile Hanks media for 2 days. The fibers
were approximately 6 mm in length and 0.8 mm in
width. They were heat-sealed as a primary seal. A
30   secondary seal was prepared by immersing the heat-
sealed ends into a polyacrylate glue which was then
crosslinked by UV-irradiation, thereby forming a
secondary seal. The sealed fibers were held at 37°C

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in HL-1 media for 2 days and then rinsed in sterile
Hanks media twice prior to implanting. The fibers
were implanted unilaterally into the striatum of the
brain of the rats for 4 weeks after which the brain
5 was histologically sectioned.

Overall biocompatibility was assessed in terms of the
fibers' interaction with brain tissue. The fibers
were compared as described in Table III for the
response to the implanted hollow fiber membranes of
10 macrophages (MACs), foreign body giant cells (FBGCs),
polymorphonuclear leukocytes (PMNs), eosinophils
(EOs), and reactive astrocytes. The p values were
calculated relative to unmodified PAN/PVC using Mann-
Whitney U-test wherein significance is set at $p \leq$
15 0.05.

TABLE III

Sample (n = 4)	MACs (p values)	FBGCs (p values)	PMNs	EOs
PAN/PVC	1.75	2	1	1
PAN/PVC-PEO-NH$_2$ (5k)	1.5 (0.75)	1 (0.05)	1	1
PAN/PVC-PEO-NH$_2$ (20k)	1.25 (0.47)	1.25 (0.16)	1	1
PAN/PVC-PEO-SC (5k)	1.25 (0.47)	1.25 (0.16)	1	1

Based on the response of reactive cells to the
implanted hollow fiber membrane in the central
nervous system and when compared to Sham surgical
25 controls with a score of 1, PAN/PVC showed average
biocompatibility with an overall rating of 1.8 (n =
4), whereas PEO-grafted PAN/PVC [PAN/PVC-PEO-NH$_2$ (5K
and 20K)] showed improved biocompatibility with an
overall rating of 1.2 (n = 8). Using the Mann-
30 Whitney U-test, a statistical difference (p values)
between PAN/PVC and PEG-grafted PAN/PVC existed for
the response of FBGCs but not for that of MACs (EOs

or PMNs). The *in vivo* experiment indicates that, qualitatively, the biocompatibility is enhanced for PAN/PVC-PEO with respect to PAN/PVC. The response of PMNs to the implanted fibers was acceptable (i.e. 1),

5   indicating that all implants were sterile and that no gross contamination was introduced during surgery. In addition, eosinophils were not found at the implant site, indicating that the membrane did not evoke an allergic response. The response of

10 immunoreactive GFAP astrocytes to the different fiber types followed the same trends described above.

Example 12:   PEO Modification of PAN/PVC Resin

A PAN/PVC resin is dissolved in an organic solvent such as dimethyl sulfoxide (DMSO), n-methyl

15 pyrrolidone (NMP) or acetone. The resin is then reacted with sodium borohydride dissolved in the same solution. The reaction results in the modification of the PAN/PVC resin by reduction of the nitrile group to an amine group. The reduced PAN/PVC-NH_2 is

20 then precipitated repeatedly in water, dried under vacuum and characterized.

Methoxy-terminated poly(ethylene oxide)-succinimidyl carbonate (PEO-SC — either 5,000 g/mole or 20,000 g/mole) is dissolved in an organic solvent as is the

25 PAN/PVC-NH_2. The PEO-SC and PAN/PVC-NH_2 are reacted together to further modify up to about 2.0% of the $-NH_2$ groups to result in the formation of PAN/PVC-PEO. Greater than 2.0% modification may change the membrane formulation properties of the

30 PAN/PVC resin. The derivatized polymer is precipitated repeatedly in water, dried and then characterized. The modified PAN/PVC, PAN/PVC-NH_2 and PAN/PVC-PEO are characterized by at least three of the following techniques: ^{1}HNMR, fluorescamine

titration, size exclusion chromatography, ion
exchange chromatography, and FTIR.

The parameters that are important for polymer
modification are polymer molecular weight and the
5   ratio of the types of monomers in the polymer. The
polymer molecular weight can be determined by a
number of techniques well known to those skilled in
the art, including gel permeation chromatography,
light scattering, and dilute solution viscosity. The
10 ratio of the types of monomers in the copolymer can
be determined by elemental analysis if the monomers
have distinct elements [e.g. PAN/PVC has nitrogen and
chlorine that distinguish each monomer] or by proton
nuclear magnetic resonance (^{1}H NMR).

15 The ratio of reagents to be used in the reactions
described above depends upon the percent conversion.
The example described below assumes 100% conversion
of both PAN/PVC to PAN/PVC—NH$_2$ and PAN/PVC—NH$_2$ to
PAN/PVC—PEO. Given that the molecular weight of
20 PAN/PVC is approximately 140,000 g/mole, and that the
PAN/PVC is approximately 45% acrylonitrile (53
g/mole) and 55% vinyl chloride (62 g/mole), there are
approximately 63,000 g/mole acrylonitrile or 1188
acrylonitrile repeat units and 77,000 g/mole vinyl
25 chloride or 1242 vinyl chloride repeat units. For a
2.0% modification with PEO, 4.4% or 53 repeat units
of acrylonitrile groups are modified. Assuming that
the percent conversion of nitrile to amine is 100%
and that of amine to succinimide is 100%, then the
30 ratio of sodium borohydride to PAN/PVC and that of
PEO—SC to PAN/VC—NH$_2$ can be determined on a molar
basis. Given that the desired percent conversion is
2.0%, 2809 g/mole of PAN/PVC are required. Thus, if
200 g (0.071 moles) of PAN/PVC are to be modified,
35 one must react 0.071 moles (2.71 g) of sodium

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borohydride with it. Similarly, with 200 g (~0.071 moles) of PAN/PVC-NH$_2$, an equal molar amount of PEO-SC is required. For 0.5% modification, 1.1% of the nitrile groups are modified. This is true for
5 PEO 5,000 and PEO 20,000. If the percent conversion of either PAN/PVC to PAN/PVC-NH$_2$ or PAN/PVC-NH$_2$ to PAN/PVC-PEO is not 100%, then the molar ratios set forth above have to be modified accordingly.

WHAT IS CLAIMED IS:

1.   A graft polymer formed by converting into intermediate reactive sites a portion of the C≡N (cyano) groups of a backbone polymer containing

5   −CH₂CH− units,
           |
          C≡N

wherein a portion of said −C≡N (cyano) groups have been converted into intermediate reactive sites, and
10   grafting polyalkylene oxide polymer chains to said backbone polymer through said reactive sites.

2.   The graft polymer of claim 1 wherein said backbone polymer is a polyacrylonitrile homopolymer.

3.   The graft polymer of claim 1 wherein said
15   backbone polymer comprises a copolymer of acrylonitrile and a monomer selected from the group consisting of vinyl chloride, vinylidene chloride, acrylic acid, butadiene-styrene, butadiene, vinyl acetate, vinyl pyridine, butadiene-co-acrylic acid,
20   maleic anhydride, and mixtures thereof.

4.   The graft polymer of claim 1 wherein said backbone polymer comprises a copolymer of acrylonitrile and vinyl chloride.

5.   The graft polymer of claim 1 in which said
25   reactive sites are formed by hydrolysis of the cyano groups.

6.   The graft polymer of claim 5 in which said reactive sites comprise -CO₂H groups which react with an amine terminal group on precursor polyalkylene
30   oxide polymer chains to form said graft polymer.

7.   The graft polymer of claim 1 in which said reactive sites are formed by reduction of said cyano groups.

8.   The graft polymer of claim 7 in which said
5   reactive sites comprise -CH$_2$NH$_2$ groups bound to said polyalkylene oxide polymer chain by reaction through a terminal reactive group thereon.

9.   The graft polymer of claim 1 in which said polyalkylene oxide comprises polyethylene oxide.

10   10.   The graft polymer of claim 1 wherein said backbone polymer is in the form of a resin and said graft polymer is in the form of a resin.

11.   The graft polymer of claim 1 wherein said backbone polymer is in the form of a permselective
15   membrane and said graft polymer is in the form of a permselective graft polymer membrane.

12.   The permselective graft polymer membrane of claim 11 in the form of a hollow fiber or flat sheet device.

20   13.   The hollow fiber or flat sheet device of claim 12 and living cells contained within said hollow fiber or flat sheet device.

14.   A method for forming a graft polymer from a polymer backbone formed by converting into
25   intermediate reactive sites a portion of the C≡N (cyano) groups of a backbone polymer containing
—CH$_2$CH— units,
          |
          C≡N
30   said method comprising:

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(a)   converting a portion of said —C≡N (cyano) groups into intermediate reactive sites, and

(b)   grafting polyalkylene oxide polymer chains to said backbone polymer through said reactive sites.

5   15.   The method of claim 14 wherein said backbone polymer is a polyacrylonitrile homopolymer.

16.   The method of claim 14 wherein said backbone polymer comprises a copolymer of acrylonitrile and a monomer selected from the group consisting of vinyl
10   chloride, vinylidene chloride, acrylic acid, butadiene-styrene, butadiene, vinyl acetate, vinyl pyridine, butadiene-co-acrylic acid, maleic anhydride, and mixtures thereof.

17.   The method of claim 14 wherein said backbone
15   polymer comprises a copolymer of acrylonitrile and vinyl chloride.

18.   The method of claim 14 in which step (a) comprises hydrolyzing said cyano group to said reactive site.

20   19.   The method of claim 18 in which said hydrolyzed reactive sites comprise —CO$_2$H groups and step (b) is performed by reacting —CO$_2$H with an amino terminal group on a precursor polyalkylene oxide polymer chain.

25   20.   The method of claim 14 in which said reactive sites are formed by reduction of said cyano group.

21.   The method of claim 20 in which said reduced reactive site is —CH$_2$NH$_2$.

22.   The method of claim 14 wherein said backbone
polymer is in the form of a resin and said graft
polymer is in the form of a resin.

23.   The method of claim 14 wherein said backbone
5 polymer is in the form of a permselective membrane
and said graft polymer is in the form of a
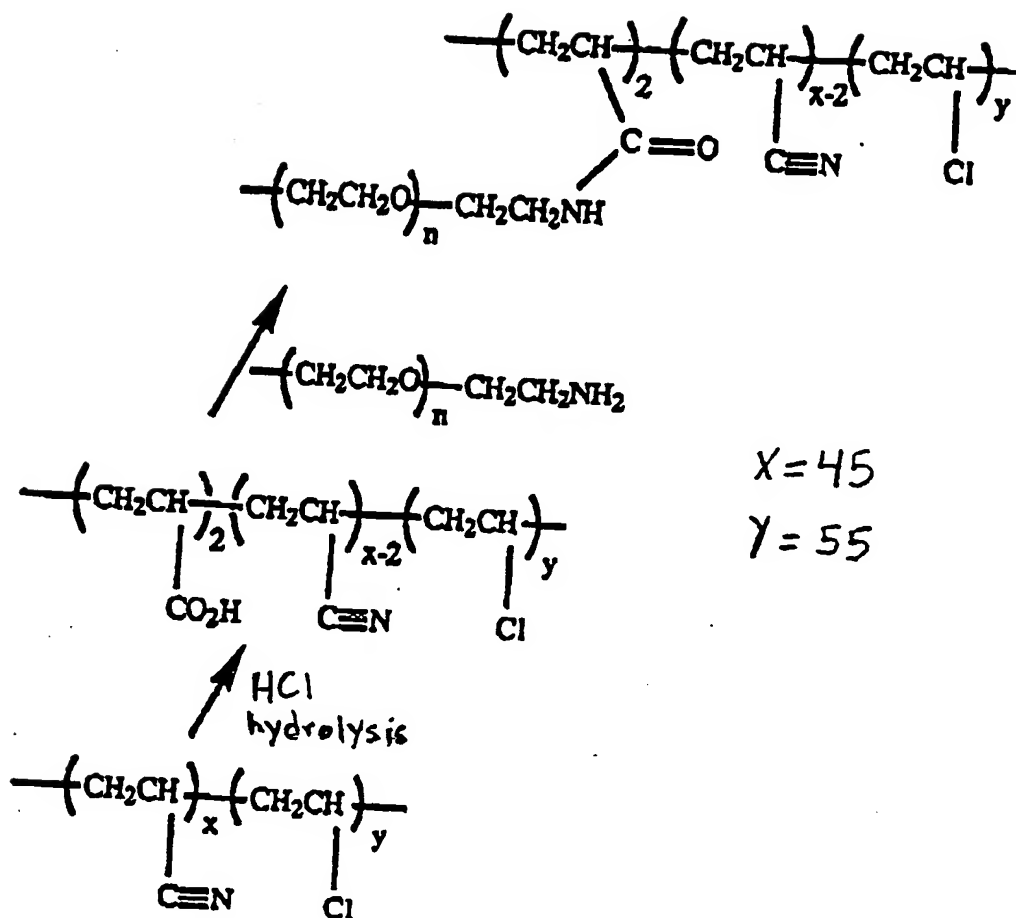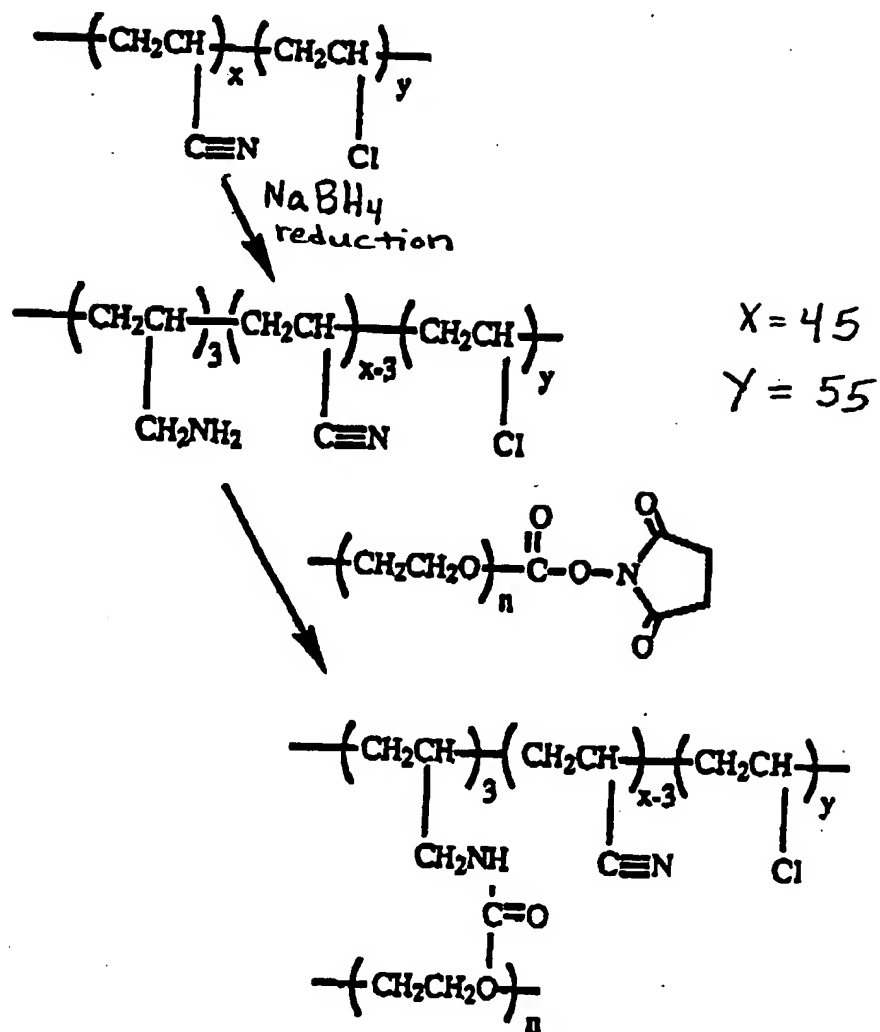permselective graft polymer membrane.

FIGURE 1

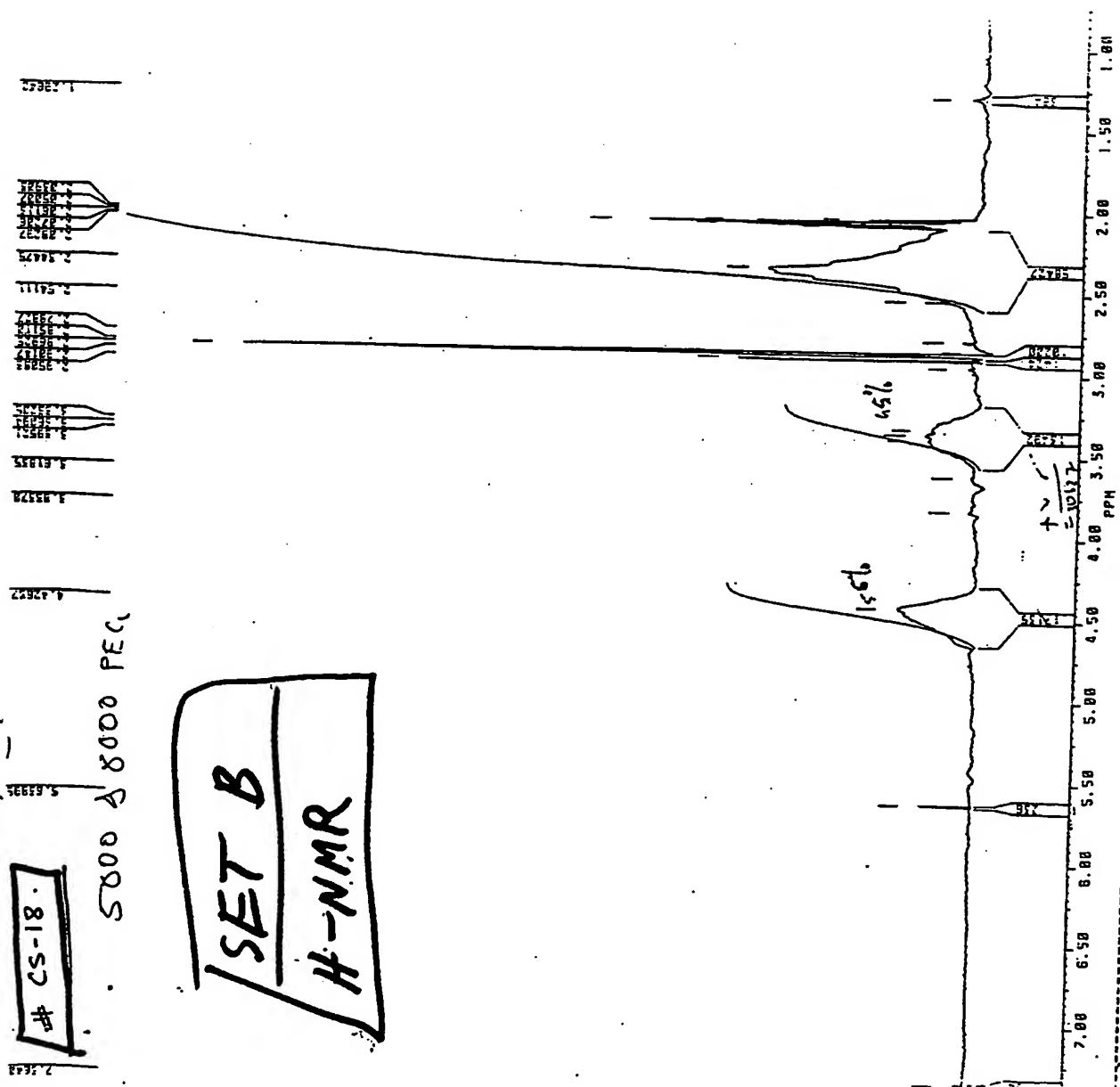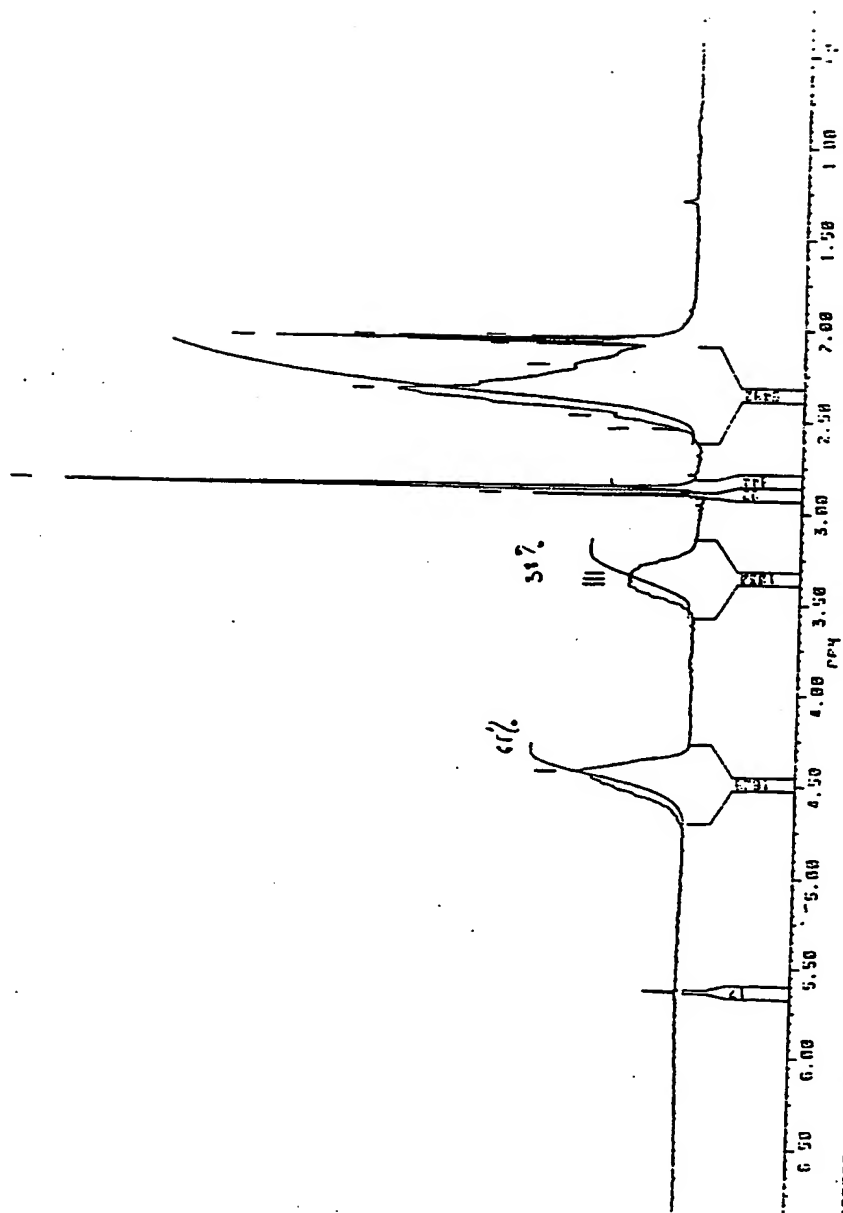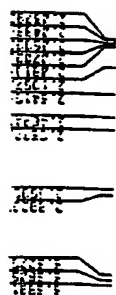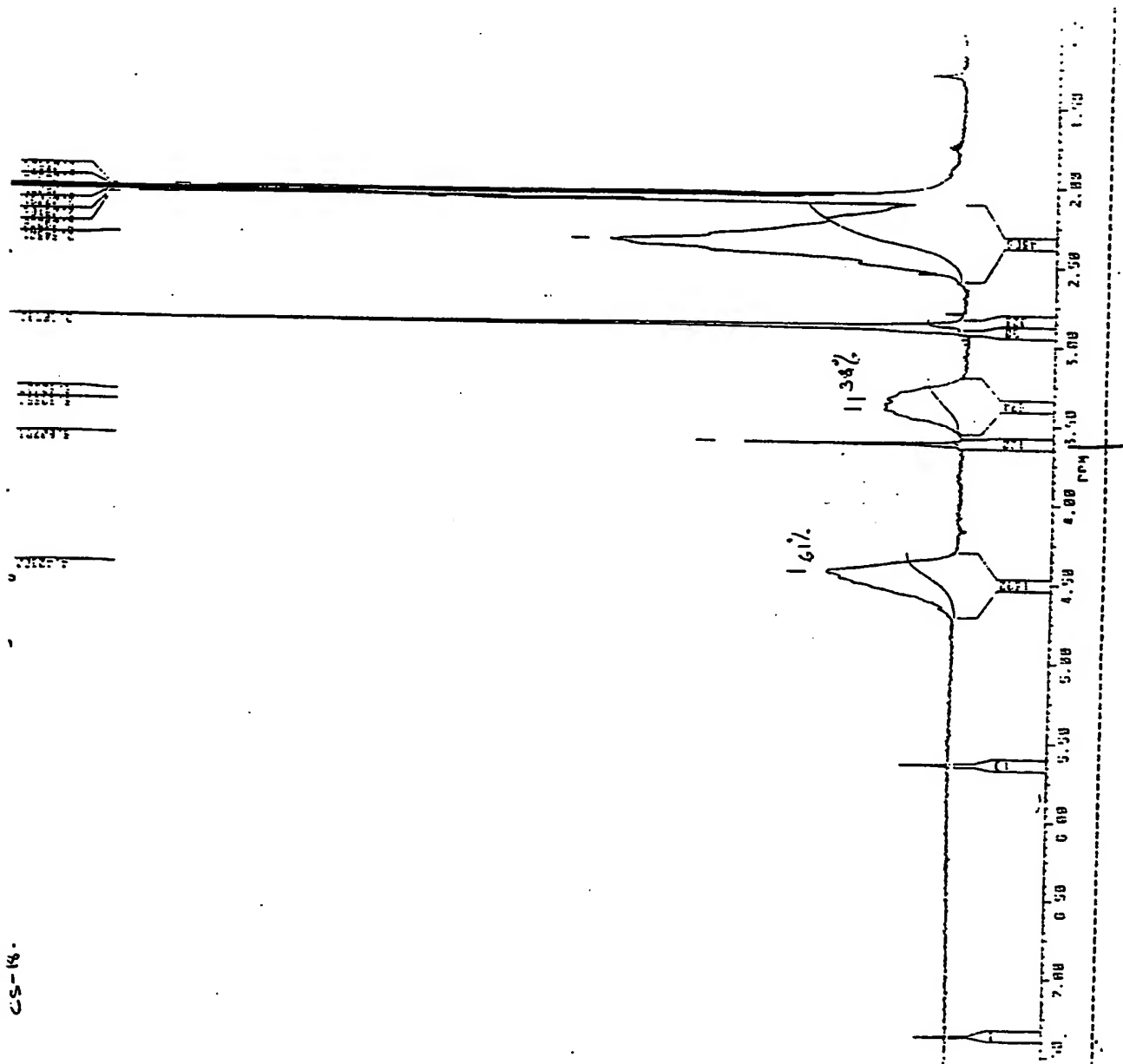$-\left(CH_2CH\right)_2\left(CH_2CH\right)_{x-2}\left(CH_2CH\right)_y$

$-\left(CH_2CH_2O\right)_n-CH_2CH_2NH-C=O$

$C\equiv N$

Cl

$-\left(CH_2CH_2O\right)_n-CH_2CH_2NH_2$

$-\left(CH_2CH\right)_2\left(CH_2CH\right)_{x-2}\left(CH_2CH\right)_y$

CO_2H $C\equiv N$ Cl

$X = 45$
$Y = 55$

HCl
hydrolysis

$-\left(CH_2CH\right)_x\left(CH_2CH\right)_y$

$C\equiv N$ Cl

FIGURE 2

$X = 45$

$Y = 55$

FIG 3

SET B
H-NMR

CS-18

B-1

F164

B-3

FIG 5

FIG 6

B-5

Fig 7
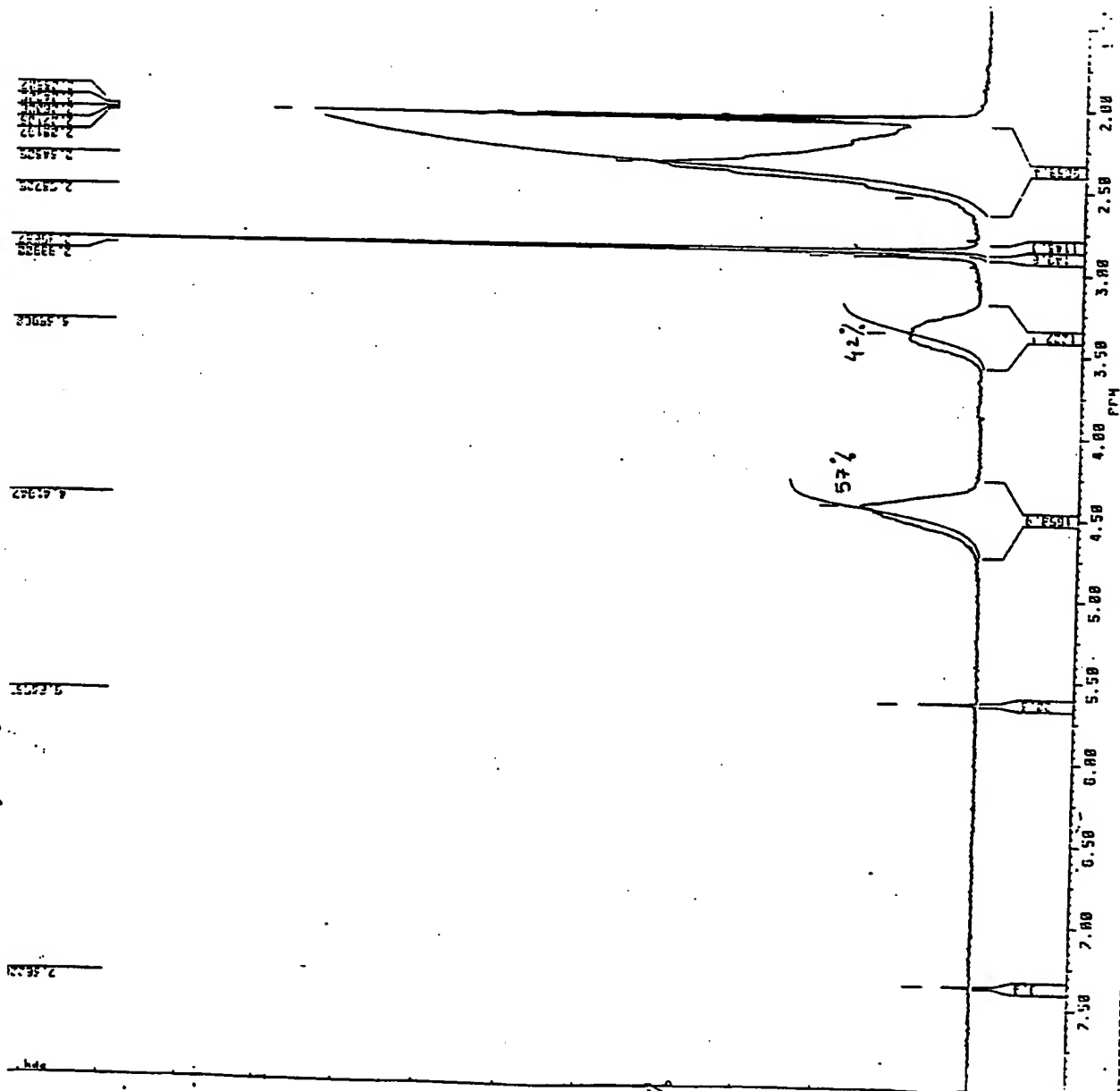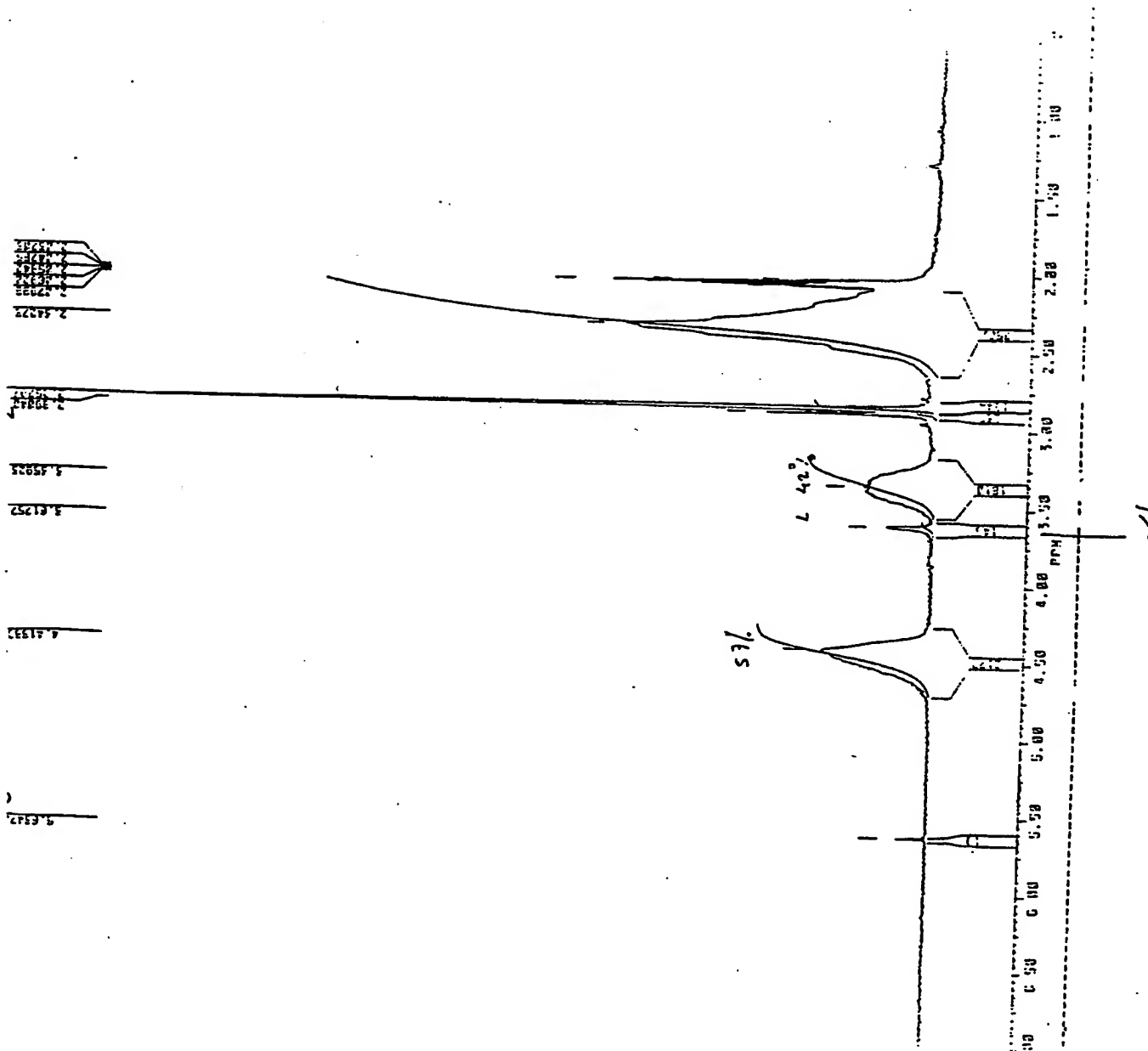
A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C08G 69/00
US CL :525/187

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 525/187, 329.1, 329.2, 385, 404, 409

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	JP, A, 63-68620 (KOKAI TOKKYO KOHO) 28 March 1988, see entire document.	1,2,9 ------------ 3-8, 10-23
Y	Shuichuli Jishu, Volume 14, No. 4, 1988, (ZHU ET AL.), "Development of PAN-PVC ultrafiltration membranes", pages 208-212.	3,4,10,16, 17
Y	US, 4,151,173 (VOGEL) 24 April 1979, see column 8.	5-8,14,15, 18-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 JULY 1994	1 2 SEP 1994

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